

Nucleic acids encoding new insect acetylcholine receptor β subunits.

The invention relates to nucleic acids encoding insect acetylcholine receptor β subunits and to polypeptides which have the biological function of such acetylcholine receptor β subunits, and in particular to their use for finding active compounds for crop protection.

Nicotinergic acetylcholine receptors are ligand-controlled ion channels which play a role in neurotransmission in the animal kingdom. The binding of acetylcholine or other agonists to the receptor causes temporary opening of the channel and allows cations to pass through. It is assumed that a receptor is composed of five subunits arranged around a pore. Each of these subunits is a protein which is composed of an extracellular N-terminal moiety, followed by three transmembrane regions, an intracellular moiety, and a fourth transmembrane region and a short extracellular C-terminal moiety. Certain subunits carry the binding site for ligands, such as acetylcholine, on their extracellular moiety. Two vicinal cysteines are a component of this binding site, and therefore a joint structural feature for all ligand-binding subunits, which are also termed α -subunits. Depending on localization and function of the receptor, subunits without this structural feature are termed β , γ , δ or ϵ subunits (Changeux et al. 1992).

Acetylcholine receptors have been the subject of many studies, in particular in vertebrates. Owing to their anatomical localization and their functional properties (conductive properties of the channel, desensitization, sensitivity to agonists and antagonists, and to toxins such as, for example, α -bungarotoxin), three groups can be distinguished. The classification correlates with the molecular composition of the receptors. There are heterooligomeric receptors with the subunit composition $\alpha_2\beta\gamma\delta$, which are found in muscle (Noda et al. 1982, Claudio et al. 1983, Devillers-Thiery et al. 1983, Noda et al. 1983a, b), heterooligomeric receptors which contain subunits from group $\alpha_2 - \alpha_6$ and $\beta_2 - \beta_4$ and which are found in the nervous system (Schoepfer et al. 1990, Heinemann et al. 1997), and homooligomeric receptors which

"Express Mail" mailing label number EL062671947US
 Date of Deposit December 3, 2000

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner of Patents and Trademarks, Washington, D.C. 20231

Dorothy P. Colangelo
 (Name of person mailing paper or fee)
 Signature of person mailing paper or fee

09732680 120800

contain subunits from group $\alpha 7 - \alpha 9$ and which are also found in the nervous system (Lindstrom et al. 1997, Elgoyhen et al. 1997). This classification is also supported when taking into consideration the relationship of the gene sequences of the various subunits. Typically, the sequences of functionally homologous subunits of different species show greater similarity than sequences of subunits from different groups, but of the same species. Furthermore, the gene sequences of all known acetylcholine receptor subunits do not just resemble each other somewhat, but also resemble those of some other, ligand-controlled ion channels (for example the serotonin receptors of the 5HT₃ type, the GABA-controlled chloride channels, the glycine-controlled chloride channels). It can therefore be assumed that all these receptors originate from a joint precursor, and they are classified in a supergene family (Ortells et al. 1995).

In insects, acetylcholine is the most important excitatory neurotransmitter of the central nervous system. Accordingly, acetylcholine receptors can be detected electrophysiologically in preparations of insect central ganglia. This is detected successfully both on post- and on presynaptic nerve endings and on the cytosomes of interneurons, motoneurons and modulatory neurons. (Breer et al. 1987, Buckingham et al. 1997). The receptors include those which are inhibited by α -bungarotoxin and those which are insensitive (Schloß et al. 1988). Moreover, the acetylcholine receptors are the molecular target for important natural (for example nicotine) and synthetic insecticides (for example chloronicotinyls).

The gene sequences of a number of insect nicotinic acetylcholine receptors are already known. Thus, the sequences of five different subunits are described for *Drosophila melanogaster* (Bossy et al. 1988, Hermanns-Borgmeyer et al. 1986, Sawruk et al. 1990a, 1990b, Schulz et al. 1998); five sequences are also described for *Locusta migratoria* (Hermesen et al. 1998), one for *Schistocerca gregaria* (Marshall et al. 1990), six for *Myzus persicae* (Sgard et al. 1998, Huang et al. 1999), two for *Manduca sexta* (Eastham et al. 1997, Genbank AJ007397) and six for *Heliothis virescens* (DE 198 19 829, Genbank AF143846, AF143847, AJ000399, AF096878, AF096879). Moreover, a series of *Drosophila melanogaster* partial gene sequences

[illegible][illegible][illegible][illegible]

nucleic acids are intended to allow the recombinant expression of nicotinic acetylcholine receptors which are composed exclusively of insect subunits.

5 The object is achieved by providing a nucleic acid comprising a sequence selected from the group consisting of

- (a) the sequence of SEQ ID NO: 1,
- 10 (b) subsequences of the sequence defined under (a) which are at least 14 base-pairs in length,
- (c) sequences which hybridize with the sequence defined under (a),
- (d) sequences which have at least 70% identity to the sequence between position
15 43 and position 1368 of the sequence defined under (a),
- (e) sequences which are complementary to the sequence defined under (a), and
- 20 (f) sequences which, owing to the degeneracy of the genetic code, encode the same amino acid sequence as do the sequences defined under (a) to (d).

25 The nucleic acids according to the invention are, in particular, single- or double-stranded deoxyribonucleic acids (DNA) or ribonucleic acids (RNA). Preferred embodiments are fragments of genomic DNA which can contain introns, and cDNAs.

A preferred embodiment of the nucleic acids according to the invention is the cDNA which has the nucleic acid sequence of SEQ ID NO: 1.

30 The degree of identity of the nucleic acid sequences is preferably determined with the aid of the program GAP from the package GCG, version 10.0, using standard settings (Devereux et al. 1984).

The term "to hybridize" as used in the present context describes the process during which a single-stranded nucleic acid molecule undergoes basepairing with a complementary strand. Starting from the sequence information disclosed herein, this
5 allows, for example, DNA fragments to be isolated from insects other than *Drosophila melanogaster* which encode polypeptides with the biological function of acetylcholine receptor β subunits.

Preferred hybridization conditions are stated hereinbelow:

10

Hybridization solution: 6X SSC/0% formamide, preferred hybridization solution: 6X SSC/25% formamide

Hybridization temperature: 34°C, preferred hybridization temperature: 42°C

15

Wash step 1: 2X SSC at 40°C,

Wash step 2: 2X SSC at 45°C; preferred wash step 2: 0.6X SSC at 55°C; especially preferred wash step 2: 0.3X SSC at 65°C.

20

The present invention encompasses nucleic acids which have at least 70% identity, preferably at least 80% identity, especially preferably at least 90% identity, very especially preferably at least 95% identity, to the sequence between position 43 and position 1368 of the sequence of SEQ ID NO: 1, preferably over a length of at least 100, especially preferably at least 500, consecutive nucleotides, and very especially
25 preferably over the entire length.

Subject-matter of the invention are furthermore vectors which contain at least one of the nucleic acids according to the invention. Vectors which can be used are all plasmids, phasmids, cosmids, YACs or artificial chromosomes used in molecular
30 biology laboratories. To express the nucleic acid according to the invention, the latter can be linked to customary regulatory sequences. The choice of such regulatory

sequences depends on whether pro- or eukaryotic cells or cell-free systems are used for expression. Especially preferred as expression control sequence are, for example, the SV40, adenovirus or cytomegalovirus early or late promoter, the lac system, the trp system, the main operator and promoter regions of the phage lambda, the fd coat protein control regions, the 3-phosphoglycerate kinase promoter, the promoter of acid phosphatase and the promoter of the yeast α -mating factor, the Baculovirus immediate early promoter, and the *Drosophila melanogaster* metallothioneine promoter.

To express the nucleic acid according to the invention, the latter can be introduced into suitable host cells. Suitable host cells are not only prokaryotic cells, preferably *E. coli*, but also eukaryotic cells, preferably mammalian or insect cells. Other examples of suitable single-celled host cells are: *Pseudomonas*, *Bacillus*, *Streptomyces*, yeasts, HEK-293, Schneider S2, SF9, CHO, COS1 and COS7 cells, plant cells in cell culture, and amphibian cells, in particular oocytes.

Subject-matter of the present invention are also the polypeptides encoded by the nucleic acid according to the invention.

Subject-matter of the present invention are also polypeptides which encompass an amino acid sequence and have at least 40% identity, preferably at least 60% identity, especially preferably at least 80% identity, to the sequence of SEQ ID NO: 2 over a length of at least 20, preferably at least 25, especially preferably at least 30, consecutive amino acids, and very especially preferably over the entire length.

The degree of identity of the amino acid sequences is preferably determined with the aid of the program GAP from the package GCG, version 10.0, using standard settings (Devereux et al. 1984).

Subject-matter of the present invention are furthermore acetylcholine receptors which encompass the polypeptides according to the invention.

The term "polypeptides" as used in the present context not only relates to short amino acid chains which are usually termed peptides, oligopeptides or oligomers, but also to longer amino acid chains which are usually termed proteins. It encompasses amino acid chains which can be modified either by natural processes, such as post-translational processing, or by chemical prior-art methods. Such modifications may occur at various sites and repeatedly in a polypeptide, such as, for example, on the peptide backbone, on the amino acid side chain, on the amino and/or the carboxyl terminus. For example, they encompass acetylations, acylations, ADP-ribosylations, amidations, covalent linkages to flavins, haem moieties, nucleotides or nucleotide derivatives, lipids or lipid derivatives or phosphatidylinositol, cyclizations, disulphide bridge formations, demethylations, cystin formations, formylations, gamma-carboxylations, glycosylations, hydroxylations, iodinations, methylations, myristylations, oxidations, proteolytic processings, phosphorylations, selenylations and tRNA-mediated additions of amino acids.

The polypeptides according to the invention may exist in the form of "mature" proteins or as parts of larger proteins, for example as fusion proteins. They can furthermore exhibit secretion or leader sequences, pro-sequences, sequences which allow simple purification, such as multiple histidine residues, or additional stabilizing amino acids.

The polypeptides according to the invention need not constitute complete acetylcholine receptor β -subunits, but may also be mere fragments thereof, as long as they can at least still exert the biological function of the complete subunits.

The polypeptides according to the invention need not be obtainable from *Drosophila melanogaster* acetylcholine receptor β subunits. Polypeptides which correspond to acetylcholine receptor β subunits of other insects, or fragments of these which can still exert the biological function of these subunits, are also considered to be in accordance with the invention.

In comparison with the corresponding region of naturally occurring acetylcholine receptor β subunits, the polypeptides according to the invention may exhibit deletions or amino acid substitutions, as long as they at least still exert the biological function of the complete subunits. Conservative substitutions are preferred. Such conservative substitutions encompass variations, one amino acid being replaced by another amino acid from amongst the following group:

1. Small aliphatic residues, nonpolar residues or residues of little polarity: ala, ser, thr, pro and gly;
2. Polar, negatively charged residues and their amides: asp, asn, glu and gln;
3. Polar, positively charged residues: his, arg and lys;
4. Large aliphatic nonpolar residues: met, leu, ile, val and cys; and
5. Aromatic residues: phe, tyr and trp.

Preferred conservative substitutions can be seen from the following list:

Original residue	Substitution
ala	gly, ser
arg	lys
asn	gln, his
asp	glu
cys	ser
gln	asn
glu	asp
gly	ala, pro
his	asn, gln
ile	leu, val
leu	ile, val
lys	arg, gln, glu
met	leu, tyr, ile

Original residue	Substitution
phe	met, leu, tyr
ser	thr
thr	ser
trp	tyr
tyr	trp, phe
val	ile, leu

The term "biological function of an acetylcholine receptor β subunit" as used in the present context means a role in generating functional acetylcholine receptors, that is, the ability of being able to interact with other subunits of the receptors.

5

A preferred embodiment of the polypeptides according to the invention is a *Drosophila melanogaster* acetylcholine receptor β subunit which has the amino acid sequence of SEQ ID NO: 2.

10

Subject-matter of the present invention are furthermore processes for producing the polypeptides according to the invention. To produce the polypeptides encoded by the nucleic acid according to the invention, host cells which contain the nucleic acid according to the invention can be cultured under suitable conditions. Thereupon, the desired polypeptide can be isolated in the customary manner from the cells or the culture medium.

15

A rapid method of isolating the polypeptides according to the invention, which are synthesized by host cells using a nucleic acid according to the invention, starts with the expression of a fusion protein, it being possible for the fusion component to be affinity-purified in a simple manner. For example, the fusion component may be glutathione S-transferase. The fusion protein can then be purified on a glutathione affinity column. The fusion component can be removed by partial proteolytic cleavage, for example on linkers between the fusion component and the polypeptide according to the invention to be purified. The linker can be designed such that it

includes target amino acids such as arginine and lysine residues, which define sites for trypsin cleavage. To generate such linkers, standard cloning methods using oligonucleotides may be employed.

5 Other purification methods which are possible are based on preparative electrophoresis, FPLC, HPLC (for example using gel filtration, reversed-phase or moderately hydrophobic columns), gel filtration, differential precipitation, ion-exchange chromatography and affinity chromatography.

10 The purification of the polypeptides according to the invention can encompass the isolation of membranes starting from host cells which express the nucleic acids according to the invention. Such cells preferably express the polypeptides according to the invention in a sufficiently high copy number, so that the polypeptide quantity in a membrane fraction is at least 10 times higher than that in comparable membranes of
15 cells which naturally express acetylcholine receptors; especially preferably, the quantity is at least 100 times higher, very especially preferably at least 1000 times higher.

The terms "isolation or purification" as used in the present context mean that the
20 polypeptides according to the invention are separated from other proteins or other macromolecules of the cell or of the tissue. The protein content of a composition containing the polypeptides according to the invention is preferably at least 10 times higher, especially preferably at least 100 times higher than in a host cell preparation.

25 The polypeptides according to the invention may also be affinity-purified without fusion component with the aid of antibodies which bind to the polypeptides.

Further subject-matters of the invention are antibodies which specifically bind to the abovementioned polypeptides or receptors. Such antibodies are produced in the
30 customary manner. For example, such antibodies may be produced by injecting a substantially immunocompetent host with such an amount of an acetylcholine receptor

polypeptide according to the invention or a fragment thereof which is effective for antibody production, and subsequently obtaining this antibody. Furthermore, an immortalized cell line which produces monoclonal antibodies may be obtained in a manner known per se. If appropriate, the antibodies may be labelled with a detection reagent. Preferred examples of such a detection reagent are enzymes, radiolabelled elements, fluorescent chemicals or biotin. Instead of the complete antibody, fragments may also be employed which have the specific binding properties desired.

The nucleic acid according to the invention can be used in particular for the generation of transgenic invertebrates. These can be employed in test systems which are based on an expression of the receptors according to the invention or variants thereof which deviate from the wild type. This also encompasses all transgenic invertebrates in which expression of the receptors according to the invention or variants thereof changes owing to the modification of other genes or gene control sequences (promoters).

The transgenic invertebrates are generated, for example, in *Drosophila melanogaster* by P-element-mediated gene transfer (Hay et al. 1997) or in *Caenorhabditis elegans* by transposon-mediated gene transfer (for example by Tc1, Plasterk 1996).

Subject-matter of the invention are therefore also transgenic invertebrates which contain at least one of the nucleic acids according to the invention, preferably transgenic invertebrates of the species *Drosophila melanogaster* or *Caenorhabditis elegans*, and their transgenic progeny. The transgenic invertebrates preferably contain the receptors according to the invention in a form which deviates from the wild type.

The nucleic acid according to the invention can be generated in the customary manner. For example, all of the nucleic acid molecule can be synthesized chemically, or else only short sections of the sequence according to the invention can be synthesized chemically and such oligonucleotides can be radiolabelled or labelled with a fluorescent dye. The labelled oligonucleotides can be used for screening cDNA libraries generated starting from insect mRNA. Clones to which the labelled

oligonucleotides hybridize are selected for isolating the DNA in question. After characterization of the DNA which has been isolated, the nucleic acid according to the invention is obtained in a simple manner.

- 5 Alternatively, the nucleic acid according to the invention can also be generated by means of PCR methods using chemically synthesized oligonucleotides.

10 The term "oligonucleotide(s)" as used in the present context denotes DNA molecules composed of 10 to 50 nucleotides, preferably 15 to 30 nucleotides. They are synthesized chemically and can be used as probes.

15 The nucleic acid according to the invention can be used for isolating and characterizing the regulatory regions which naturally occur in the vicinity of the coding region. Such regulatory regions are thus also subject-matter of the present invention.

20 The nucleic acid according to the invention allows new active compounds for crop protection or pharmaceutical active compounds for the treatment of humans and/or animals to be identified, such as compounds which alter the conductive properties of the acetylcholine receptors according to the invention as modulators, in particular as agonists or antagonists. To this end, a recombinant DNA molecule comprising the nucleic acid according to the invention is introduced into a suitable host cell. The host cell is grown in the presence of a compound or a sample comprising a variety of compounds under conditions which allow expression of the receptors according to the invention. A change in receptor properties can be detected as described hereinbelow in
25 Example 2. This allows insecticidal substances to be found.

30 Also, the nucleic acid according to the invention allows compounds to be found which bind to the receptors according to the invention. These too may be employed as insecticides. For example, host cells which contain the nucleic acid according to the invention and express the receptors or polypeptides in question or the gene products themselves are contacted with a compound or a mixture of compounds under

09732680 120800

conditions which allow the interaction of at least one compound with the host cells, the receptors or the individual polypeptides.

5 Using host cells or transgenic invertebrates which contain the nucleic acid according to the invention, it is also possible to find substances which alter receptor expression.

The nucleic acid according to the invention, vectors and regulatory regions described hereinabove can also be used for finding genes which encode polypeptides which participate in the synthesis, in insects, of functionally similar acetylcholine receptors.
10 Functionally similar receptors are to be understood as meaning, in accordance with the present invention, receptors which comprise polypeptides which, while differing from the amino acid sequence of the polypeptides described herein, essentially have the same functions.

15 **Information on the sequence listing and the figure**

SEQ ID NO: 1 shows the nucleotide sequence of the isolated Db3-cDNA, starting with position 1 and ending with position 1539. SEQ ID NO: 1 and SEQ ID NO: 2 furthermore show the amino acid sequences of the protein derived from the
20 Db3-cDNA sequence.

SEQ ID NO: 3 and SEQ ID NO: 4 show the oligodeoxynucleotides described in Example 1.

25 Fig. 1 shows the acetylcholine-induced currents measured on *Xenopus* oocytes with the aid of whole-cell discharges plotted against time. Currents are shown in nano-ampere, time in seconds. The oocytes had been injected with cDNA expression plasmids which encoded the *Drosophila* $\alpha 1$, $\alpha 2$ and $\beta 3$ subunits. The timings of the acetylcholine applications are identified with transverse bars.

30

Examples

Example 1

- 5 Isolation of the above-described polynucleotide sequence

General

Polynucleotides were manipulated by standard methods of recombinant DNA technology (Sambrook et al., 1989). Nucleotide and protein sequences were
10 processed in terms of bioinformatics using the package GCG version 10.0 (GCG Genetics Computer Group, Inc., Madison Wisconsin, USA).

Isolation of partial polynucleotide sequences by means of PCR

- 15 Based on a database search with the protein sequence of the *Drosophila melanogaster* ARD subunit versus the genomic *Drosophila melanogaster* database, a nucleic acid region was identified which has 28% identity to ARD at the amino acid level. Oligodeoxynucleotide primers (dg1sense: 5'-TGGCARCCITCICARTAYGA-3', dg2anti: 5'-CATRATYTTYTCICCCICCCAT-3') were derived on the basis of this
20 partial sequence. RNA was isolated by means of Trizol reagent (Gibco BRL) from *Drosophila melanogaster* embryos following the manufacturer's instructions. 10 µg of this RNA were employed in a cDNA first-strand synthesis (Superscript preamplification system for cDNA first-strand synthesis, Gibco BRL, following the manufacturer's instructions, reaction temperature 45°C). Then, in each case 1/100 of
25 the abovementioned first-strand cDNA was employed in a polymerase chain reaction (PCR) with the oligonucleotides dg1sense and dg2anti (Taq DNA polymerase, recombinant, Gibco BRL). The PCR parameters were as follows: 94°C, 1 minute; 35 times (94°C, 30 s; 55°C, 30 s; 72°C, 45 s). This resulted in an approx. 0.6 kb band which was discernible in the agarose gel (1%). The band was subcloned by means of
30 the pCR TOPO kit (Invitrogen).

Isolation of poly-A-containing RNA from *Drosophila melanogaster* tissue, and construction of the cDNA libraries.

5 The RNA for the cDNA library was isolated from *Drosophila melanogaster* embryos and larvae using Trizol (Gibco BRL) following the manufacturer's instructions. The poly-A-containing RNAs were now isolated from this RNA by purification over Dyna Beads 280 (Dyna). 5 µg of these poly-A-containing RNAs were subsequently employed in the construction of the cDNA library using the λ-ZAP-CMV vector (cDNA Synthesis Kit, ZAP-cDNA Synthesis Kit and ZAP-cDNA Gigapack III Gold Cloning Kit, all from Stratagene).

Isolation of the complete polynucleotide sequences from the cDNA library

15 Screening with 10^6 plaque-forming units was carried out with the aid of the DIG system (all reagents and consumables from Boehringer Mannheim, following instructions in "The DIG System User's Guide for Filter Hybridization", Boehringer Mannheim). The DNA probe employed was prepared by PCR by means of digoxigenin-labelled dUTP. The hybridizations were performed overnight at 42°C in DIG Easy Hyb (Boehringer Mannheim). Labelled DNA on nylon membranes was detected by chemoluminescence (CDP-Star, Boehringer Mannheim) using X-ray films (Hyperfilm MP, Amersham). Plaques which had been singled out and which were positive in the hybridization were transferred to plasmids (pCMV) by means of in-vivo excision (Stratagene, ZAP-cDNA Synthesis Kit). For identification, the plasmids isolated were subjected to incipient sequencing by means of T3 and 25 T7 primers (ABI Prism Dye Terminator Cycle Sequencing Kit, ABI, with ABI Prism 310 Genetic Analyzer). The complete polynucleotide sequences of DB3 were determined by primer walking by means of the Cycle Sequencing ABI Prism Dye Terminator Cycle Sequencing Kit, ABI, with ABI Prism 310 Genetic Analyzer.

09732680 120800

Example 2

Expression in *Xenopus* oocytes of recombinant insect acetylcholine receptors containing the new *Drosophila* $\beta 3$ subunit.

5

Oocytes were injected simultaneously with cDNA expression plasmids which encoded the *Drosophila* $\alpha 1$, $\alpha 2$ and $\beta 3$ subunits. The α subunits were cloned into pcDNA3, the β subunit into pCMV, as described above. After incubation for three to five days, the currents through the oocyte membrane were measured as described using whole-cell discharges (Cooper et al. 1996). To this end, the potential difference over the cell membrane was kept constant at -80mV and the cells were stimulated with acetylcholine (10 μ M). Immediately after the stimulus, strong inward currents were measured, which were typical of the activation of ion channels (Fig. 1). This demonstrates that the new *Drosophila* $\beta 3$ subunit forms functional receptors with one of the two coinjected α subunits, or with both.

10

15

003021" 089226/60

[illegible]

- [illegible]

- Matsuda et al. (1998), Effects of the α subunit on imidacloprid sensitivity of recombinant nicotinic acetylcholine receptors, Br. J. Pharmacol. 123, 518-524
- Noda et al. (1982), Primary structure of α -subunit precursor of *Torpedo californica* acetylcholine receptor deduced from cDNA sequence, Nature 299, 793-797
- Noda et al. (1983a), Primary structures of β - and δ -subunit precursor of *Torpedo californica* acetylcholine receptor deduced from cDNA sequences, Nature 301, 251-255
- Noda et al. (1983b), Structural homology of *Torpedo californica* acetylcholine receptor subunits, Nature 302, 528-532
- Ortells et al. (1995), Evolutionary history of the ligand-gated ion-channel superfamily of receptors, Trends in Neuroscience 18, 121-127
- Plasterk (1996), The Tc1/mariner transposon family, Transposable Elements/Current Topics in Microbiology and Immunology 204, 125-143
- Sambrook et al. (1989), Molecular Cloning, A Laboratory Manual, 2nd ed. Cold Spring Harbor Press
- Sawruk et al. (1990a), Heterogeneity of *Drosophila* nicotinic acetylcholine receptors: SAD, a novel developmentally regulated α -subunit, EMBO J. 9, 2671-2677
- Sawruk et al. (1990b), SBD, a novel structural subunit of the *Drosophila* nicotinic acetylcholine receptor, shares its genomic localization with two α -subunits, FEBS Lett. 273, 177-181

- Schloß et al. (1988), Neuronal acetylcholine receptors of *Drosophila*: the ARD protein is a component of a high-affinity α -bungarotoxin binding complex, *EMBO J* 7, 2889-2984
- 5 Schoepfer et al. (1990), Brain alpha-bungarotoxin binding protein cDNAs and MAbs reveal subtypes of this branch of the ligand-gated ion channel gene superfamily, *Neuron* 5, 35-48.
- Schulz et al. (1998), D α 3, a new functional α -subunit of nicotinic acetylcholine
10 receptors from *Drosophila*, *J. Neurochem.* 71, 853-862
- Sgard et al. (1998), Cloning and Functional Characterization of Two Novel Nicotinic Acetylcholine Receptor α subunits from the Insect Pest *Myzus persicae*; *J. Neurochem* 71, 903-912